Very Fast Kinetics

- What is “very fast?”
  - Stopped flow methods: 1+ ms^{-1} rates
  - What about 10-100 μs^{-1} rates?

- Examples:
  - Very fast protein folding
  - pH neutralization
  - Some ion binding (e.g. Ca^{2+})

Fluctuation Methods

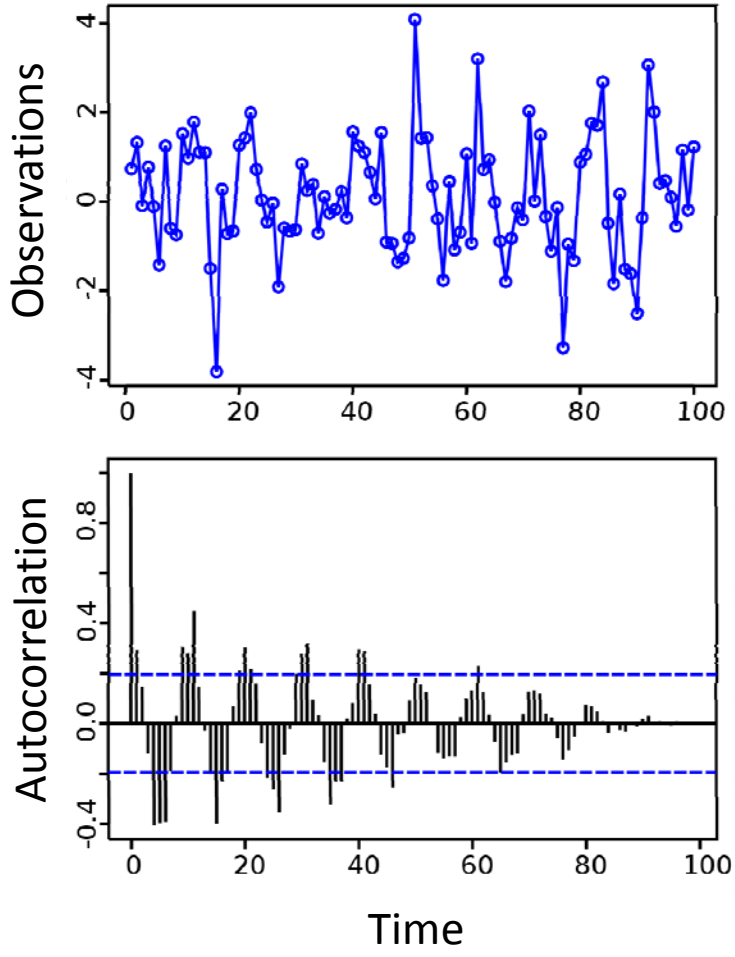
- **Idea:** Measure some quantity as it changes over time
  - System is at equilibrium, but changes still occur
  - Fluctuations will reflect kinetics

- **Challenges:**
  - Need sensitive equipment to measure small changes
  - Need a system that is theoretically (and practically) tractable

Fluctuation Methods: Examples

- Methods
  - Refractive index
  - Concentration
  - Light scattering
  - Pressure

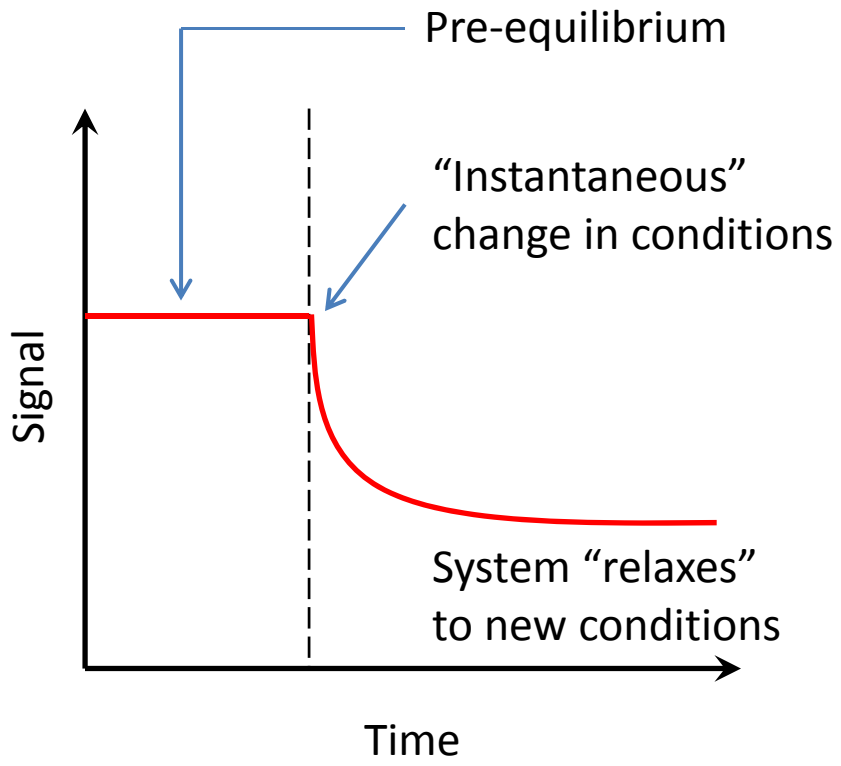
- **Autocorrelation:** how an observed quantity correlates to itself (typically over time)



"Autocorrelation." *Wikipedia.* Accessed 11/8/2012.

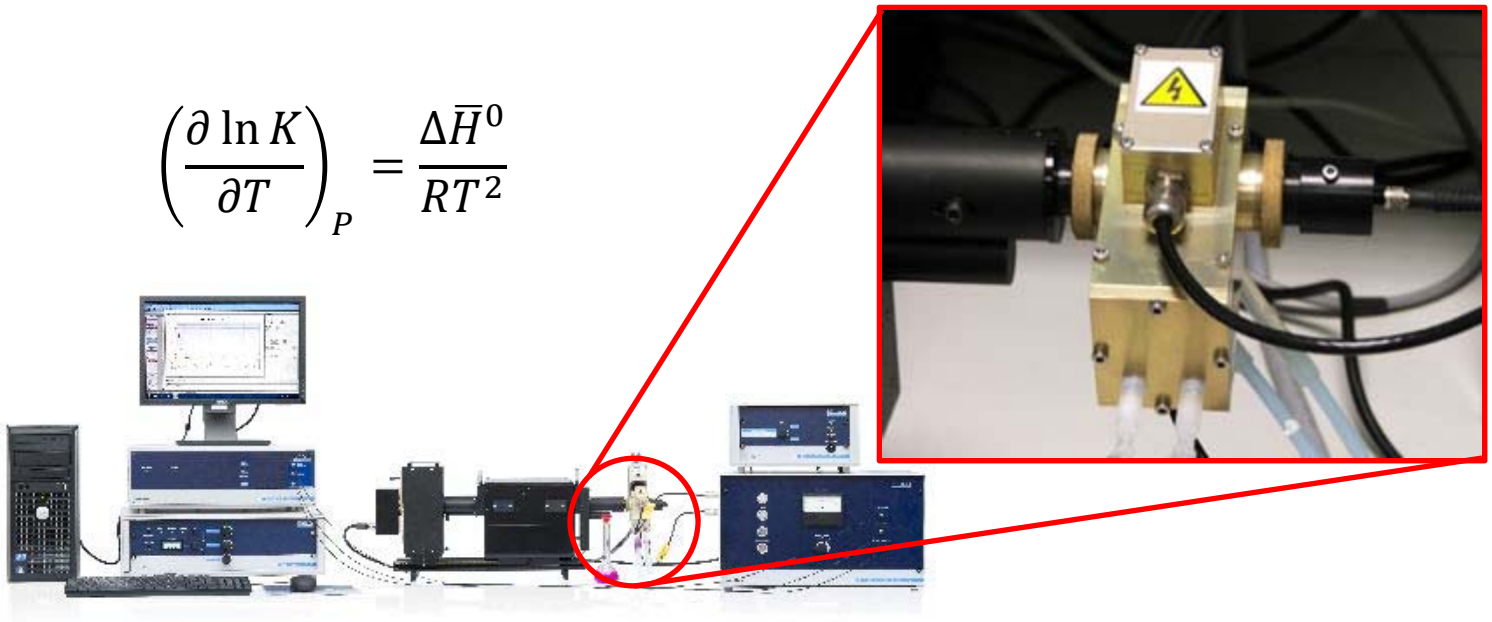
Relaxation/Perturbation Methods

- **Idea:** Start at equilibrium, then change conditions (no new intermediates)
- New equilibrium will be established → observe change over time
- **Critical:** Kinetics will reflect *new* conditions, not old conditions



Method 1: Temperature Jump

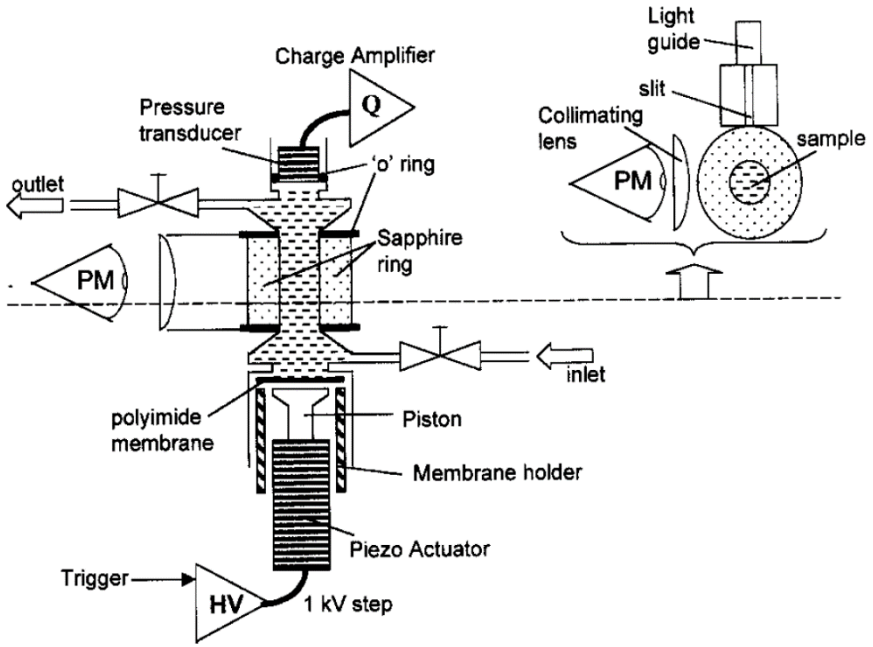
$$\left(\frac{\partial \ln K}{\partial T}\right)_P = \frac{\Delta \bar{H}^0}{RT^2}$$



- Electrical discharge, laser pulse rapidly increases temperature (small sample volume)

Image Source: TgK Scientific www.hi-techsi.com

Method 2: Pressure Jump



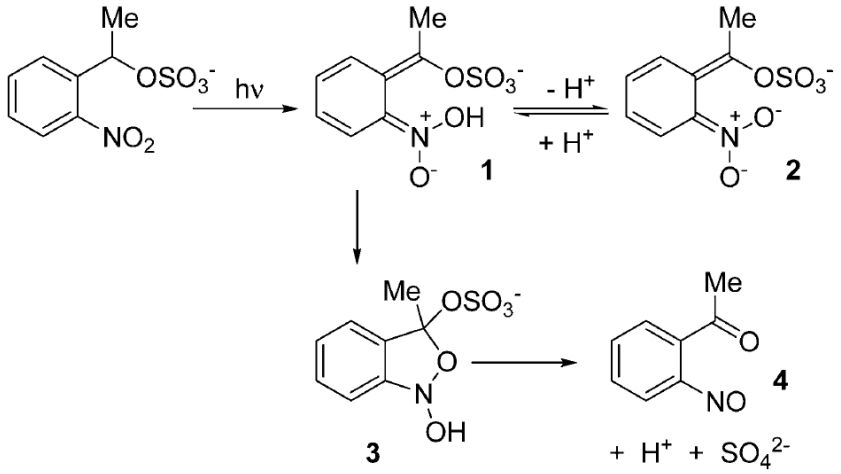
$$\left(\frac{\partial \ln K}{\partial P}\right)_T = -\frac{\Delta \bar{V}^0}{RT}$$

- Diaphragm bursts to increase (or decrease) pressure of system
- To keep change in K small, $\Delta P \approx 10^2 - 10^3$ atm

Pearson, D. S., *et al*. (2002) *Biochem. J.* **366:** 643.

Method 3: Flash Photolysis

Scheme 1. Proposed Reaction Mechanism for Photolysis of 1-(2-Nitrophenyl)ethyl Sulfate[14]



- Rapid flash of light (or laser pulse) releases H+ to change pH
- Other "caged" molecules exist (e.g. ATP)

Abbruzzetti, S., *et al.* (2005) *JACS*. **127:** 9865.

Relaxation Kinetics Example

- Binding reaction:

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} P$$

Why Can We Ignore x^2?

(or ΔP^2)

- Consider equilibrium:
  - Let A_{eq} = 10 mM and B_{eq} = 50 mM
  - If small perturbation, x is 1 mM

- Then:
  - $A_{eq}B_{eq} = 500\ \mathrm{mM}^2$
  - $\left(A_{eq} + B_{eq}\right)x = 60\ \mathrm{mM}^2$
  - $x^2 = 1\ \mathrm{mM}^2$ ← This is much smaller than the other two

Implications of Relaxation Kinetics

- Remember kinetic rates (and equilibrium) concentrations are from *new* conditions!!

- Curves will always be exponential, because x^2 will *always* be small if perturbation is small

- Determining τ at multiple conditions (e.g. A_{eq}, B_{eq} and P_{eq}) can reveal rate constants

Relaxation Time Constants

TABLE 7.4 Relaxation Times for Reactions Involving Single Steps

Mechanism	Relaxation time*
$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$	$\tau = \dfrac{1}{k_1 + k_{-1}}$
$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$	$\tau = \dfrac{1}{k_{-1} + k_1([\overline{A}] + [\overline{B}])}$
$A + B + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$	$\tau = \dfrac{1}{k_{-1} + k_1([\overline{A}][\overline{B}] + [\overline{B}][\overline{C}] + [\overline{A}][\overline{C}])}$
$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P + Q$	$\tau = \dfrac{1}{k_1([\overline{A}] + [\overline{B}]) + k_{-1}([\overline{P}] + [\overline{Q}])}$
$2A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A_2$	$\tau = \dfrac{1}{4k_1[\overline{A}] + k_{-1}}$

*$[\overline{A}]$, $[\overline{B}]$, etc., represent the equilibrium concentrations after the perturbation.
Source: M. Eigen and L. De Maeyer, in *Investigation of Rates and Mechanisms of Reactions,* 3d ed., vol. 6, part 2, ed. G. G. Hammes (New York: Wiley-Interscience, 1974), chapter 3.

Tinoco, et al. p. 351.

Example: Dimerization Kinetics in DNA

- Dimerization scheme:

$$2\mathrm{A} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} A_2$$

- Time constant:

$$\tau = \frac{1}{4k_1 A_{eq} + k_{-1}}$$

- Modified time constant:

$$\frac{1}{\tau^2} = k_{-1}^2 + 8k_1 k_{-1} A_{total}$$

Strategy: Relaxation Kinetics

- For individual points (to determine τ):
  - *Always* first-order kinetics (exponential)
  - Plot $\ln[A]$ vs. t → slope will be $\pm\frac{1}{\tau}$

- Determine how τ varies with A_{eq}, B_{eq}, etc.
  - Figure out how to linearize the function to relate slope, intercept to kinetic parameters

- **Remember:** Linearization is *bad* for real-life situations; use nonlinear least squares